Long-Term Storage of *Ustilago tritici*

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ABSTRACT

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An extensive collection of isolates of *Ustilago tritici* from around the world is maintained at the Cereal Research Centre. As the collection size increases, however, the time and effort needed to maintain the collection becomes greater, as does the need for a good technique for long term storage of *U. tritici*. Race T2 of *U. tritici* was inoculated to spikelets of wheat in February 1976. The matured inoculated heads were thrashed and the seed stored in a desiccator with silica-gel at -15°C . Every 2 years, 60 seeds were removed and planted to determine viability and proportion of infected adult plants. Between 57 and 83% of the seed produced adult plants, and the percent of infected plants ranged between 56 and 98% during the 20 years. There was no significant change in seed germination over time, but there was a positive relationship ($P < 0.0664$, $R^2 = 0.452$) between the time of storage and the arcsine of the proportion of smutted plants. Storage of *U. tritici* in infected seed at -15°C and low relative humidity is an efficient method for long-term storage of this fungus.

Hanna and Popp (3) were the first researchers to demonstrate the existence of races of *Ustilago tritici* (Pers.) Rostr. in North America. Since that time, smut pathologists at the Cereal Research Centre (CRC), Winnipeg, Manitoba, have screened many different isolates of the fungus to determine the race composition of natural populations of *U. tritici* in western Canada and other parts of the world (2,4,6,8). This work has been important in aiding wheat breeders to determine which resistance genes are necessary for incorporation into new wheat lines, and allowing plant pathologists to study virulence shifts in natural pathogen populations. The collected races of *U. tritici* have also been useful in genetic studies of the host and pathogen (5). Unfortunately, as new races of the fungus are found and as collections of isolates of similar race from different parts of the world get larger, it becomes

laborious and time consuming to preserve the collections.

Wheat spikes with sori of the different *U. tritici* races are usually dried and stored at the CRC in glass jars at 2 to 4°C (4). Spores stored in such a manner can survive at acceptable germination rates (greater than 50%) for periods up to 16 years (Table 1), but the results are variable. After storage in glass jars for 16 years, races such as T17 and T18 had germination rates of 60 to 80%, while races such as T7, T8, and T10 had germination rates of less than 1 to 5%. Race T13 had a germination rate of only 10% after 11 years of storage in glass jars, and races T4 and T26 had germination rates of 45 and 50%, respectively, after only 7 years of storage, suggesting that they should be rejuvenated soon. In general, teliospores of *U. tritici* and other smut fungi stored in glass jars at 2 to 4°C should survive for at least 5 years (4,12), but one cannot be confident of good spore survival beyond 10 years of storage in this manner. Since host reinfection and subsequent increase of teliospores of an isolate or race of *U. tritici* is laborious and time consuming, a longer-term storage technique is desirable.

The long-term storage of isolates of *Ustilago* spp. as mycelium in infected seed has been previously suggested. Tapke (13) found that barley seed infected with *U. nuda* (C.N. Jensen) Rostr. (Syn. *U. nuda* (C.N. Jensen) Kellerm. & Sw.) and stored at -2 to 0°C was viable after 7 years of storage, and 73% of the plants grown from

the seed produced sporulating sori (for simplicity, such plants will be termed "infected"). Russell (11) stored seed infected with *U. nuda* in glass jars at room temperature and found that some samples showed a reduction in the percent of infected plants grown from the seed after 6 years of storage, while others showed little reduction until the samples were in storage for 10 years. This decline was attributed to reduced germination of infected seed because of weaker embryos (11). Roszko et al. (10) found that mycelium of *U. nuda* in infected seed survived only a short period of time and concluded that in order to obtain a sufficiently viable inoculum with simultaneously high seed germination, the period of storage should not exceed 2 years. In addition, the percent infection of the host seed decreased with longer storage. Tiemann (15) observed that the level of infection decreased only slightly in plants grown from seed infected with *U. tritici* which had been stored at room temperature for 3 years. Perhaps more importantly, Buchheim (1) found that storage of wheat seed below 0°C had no effect on the level of loose smut infection in the plants grown from such seed. The findings of Buchheim along with the prediction that the viability of wheat seed stored at -20°C and 5% moisture content would drop only 5% in 78 years (9) prompted us to develop a technique for the long term preservation of isolates of *U. tritici*.

MATERIALS AND METHODS

Race T2 (4) of *U. tritici* was inoculated to spikelets of the wheat differential TD-3 (Florence $\times$ Aurore; 4) in February 1976, using a hypodermic needle and syringe and an inoculum concentration of approximately 1 g of teliospores/liter water (4,7). The matured inoculated heads were thrashed, and the seed was placed in a desiccator with silica-gel and kept at room temperature for one week. During this period, 60 seeds were removed and planted in soil beds in the greenhouse to determine the percent germination of the seed and the percent infected plants. The desiccator and remaining seed then were placed in a freezer at -15°C for storage. Every 2 years, 60 seeds were removed from storage and planted in soil beds in the greenhouse to

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Fig. 1. The effect of long-term storage at -15°C of wheat seed (differential TD-3 [Florence $\times$ Aurore]) infected with race T2 of *Ustilago tritici* on the subsequent proportion of plants with smutted heads arising from the seed ($P < 0.0664$, $R^2 = 0.452$).

determine the seed viability and proportion of infected adult plants.

RESULTS AND DISCUSSION

The results are presented in Table 2 (a summary of the first 10 years of the study has been previously reported; 4). Between 57 and 83% of the seed produced adult plants, and the percent of infected plants ranged between 56 and 98% during the 20 years. Unlike the results of previous studies (10,11,13,15), the proportion of smutted plants among the surviving seed appeared to be higher at the later sampling dates. The data were analyzed using regression analysis comparing the time of storage with the number of emerged plants, the number of smutted plants, and arcsine transformations of the square roots of the proportion of emerged plants/seed planted and the proportion of smutted/healthy plants. There was a positive relationship (P

< 0.0664 , $R^2 = 0.452$) between the time of storage and the arcsine transformation of the square root of the proportion of smutted/healthy plants (Fig. 1), but not between the time of storage and the other parameters. If this relationship is real, the mechanism for the increase in the proportion of smutted heads is not clear, but the survival and/or growth of *Ustilago tritici* within infected seed or germinating seedlings may be enhanced by the cold storage treatment.

Levels of smutted plants from infected seed are notoriously variable (14), probably because of the early stages of plant growth being affected by uncontrollable variables in soil beds in greenhouses or the field. The variability in infection levels observed in Table 2 probably reflects the variability that is normally observed after growing inoculated seed. It is expected that the percentage of infected plants will remain high (greater than 50%) for decades. It should be noted that this experiment is ongoing, and it is hoped that we will be able to collect data on storage of T2 for another 30 to 40 years. A larger population of infected seed samples would have enabled replication and a more critical examination of germination/infection levels. However, the population used is adequate to illustrate the eventual decline in germination/infection over a long time period, and allows future investigators to minimize the resources needed to continue this work. Currently, in order to conserve labor and storage facilities, all races of *U. tritici* and *U. nuda* are being stored in this manner at the CRC. Long term storage at -15°C of the different races and isolates of these pathogens in infected seed consumes much less time and space than traditional storage techniques.

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Table 1. Germination^a of teliospores of different races of *Ustilago tritici* stored at 2 to 4°C in glass jars

Race of <i>Ustilago tritici</i>	Date smutted heads harvested	Percent germination of teliospores
T1	April 1980	10
T7	April 1980	< 1
T8	November 1980	1
T10	November 1980	5
T17	November 1980	60
T18	November 1980	80
T2	May 1981	5
T15	June 1981	1
T27	February 1981	80
T19	January 1982	5
T28	January 1982	80
T30	January 1982	20
T35	January 1982	40
T25	September 1983	60
T6	December 1985	80
T16	April 1985	40
T13	April 1985	10
T3	1989	85
T4	1989	45
T26	1989	50
T22	May 1990	90
T9	June 1993	85

^a Percent germination was determined on half-strength potato dextrose agar in January 1996.

Table 2. Percentage of plants produced and subsequent percentage of smutted plants from 60-seed samples of wheat differential TD-3 (Florence $\times$ Aurore) inoculated with race T2 of *Ustilago tritici* and stored at -15°C since 1976

Number of years in storage	Percentage of seed that produced adult plants	Percentage of adult plants infected by <i>Ustilago tritici</i>
0	57	56
2	82	86
4	80	73
6	65	74
8	83	84
10	77	80
12	82	84
14	82	59
16	75	91
18	67	98
20	78	91

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